IgG Antibody to Toxoplasma Gondii

ELISA

Enzyme Immunoassay for the qualitative determination of IgG antibodies to Toxoplasma gondii in human serum or plasma.

Intended use
The anti-Toxo (IgG) ELISA is intended for the qualitative detection of IgG antibodies to Toxoplasma gondii in human serum or plasma.

Introduction
Toxoplasma gondii is a coccidian parasite initially isolated in 1908 from a North African rodent - the gondii. Since then, the organism has been found in many species of birds, reptiles and mammals. Man is infected with Toxoplasma gondii from various suspected sources: ingestion of infected meat, especially mutton and pork, or ingestion of soil contaminated by oocysts from domestic and feral cat. Transmission by organ transplant, transfusion or activation of quiescent infections is also documented. Congenital Toxoplasmosis is a disease with an extraordinarily wide range of manifestations; so wide in fact, that it must be considered in the differential diagnosis of nearly all types of obscure illness occurring during infancy.

Because symptoms are sometimes nonspecific (i.e., anemia, splenomegaly, jaundice, fever, hepatomegaly, adenopathy and vomiting), congenital Toxoplasmosis is easily misdiagnosed on the clinical grounds, even in sick infants who have the generalized form of the disease. Toxoplasmosis must also be considered in the differential diagnosis in any immunosuppressed patient who has clinical or laboratory evidence of damage to the central nervous system. The organism is one of the most common latent infectious agents of man throughout the world.

The sensitivity, specificity, and reproducibility of enzyme-linked immunosorbent assays is comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radio immunoassays.

Principle of the test
anti-Toxo (IgG) ELISA is based on indirect ELISA. Microwells are pre-coated with Toxo antigens. Once the sample is added, anti-Toxo (IgG), if present, binds to pre-coated antigens. After incubation and wash procedures, enzyme conjugate reagent is added, and the anti-human IgG inside binds to anti-Toxo(IgG) attached to the solid phase in the previous step. After another incubation and wash procedures, add substrate solution and chromogen solution to initiate a chromogenic reaction. Once the color development is completed, add the stop solution, and then read the absorbance of each sample. The color intensity is directly proportional to anti-Toxo (IgG) concentration.

Storage of Test Kits and Instrumentation
1. Unopened test kit should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date. Thawed samples must be mixed prior to testing.
2. Opened test kit will remain stable until the expiration date shown, provided it is stored as prescribed above.

Specimen Collection and Preparation
1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

Materials

Materials provided
1. Coated Wells: microplate with Toxo antigen coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: horseradish peroxidase (HRP) labeled anti-human (IgG) in stabilizing buffer (1 vial, 11.5 ml).
3. Negative Control: human serum/plasma not reactive for anti-Toxo IgG, diluted in buffer with preservatives (1 vial, 1.0 ml).
4. Positive Control: human serum/plasma reactive for anti-Toxo IgG, diluted in buffer with preservatives (1 vial, 1.0 ml).
5. Wash Fluid Concentrate: PBS-Tween (1 bottle, 50.0 ml, 20×).
6. Substrate Solution: hydrogen peroxide (1 vial, 7.5 ml).
7. Chromogen Solution: 3, 3’, 5, 5’-tetramethylbenzidine (TMB) (1 vial, 7.5 ml).
8. Stop Solution: 1.0 M H2SO4 (1 vial, 7.5 ml).
9. Sample Diluent: buffer solution with preservatives (1 bottle, 11.5 ml).

Materials required but not provided
1. Distilled water.
2. Precision pipettes: 0.05ml, 0.1ml, 1.0ml.
3. Disposable pipette tips.
4. Microtiter well reader.
5. Vortex mixer or equivalent.
6. Absorbent paper.
7. Graph paper.

Reagent Preparation
1. All reagents should be allowed to reach room temperature (18-25°C) before use.
Obtain the assays from the fridges. Place at room temperature (18 – 25°C) and equilibrate for at least 30 minutes.

2. Mix the reagents by gently inverting or swirling.
3. Dilute Wash Fluid Concentrate 20 folds with distilled water.
4. Calibrate the temperature of the incubator at 37°C. Only use after the temperature is stabilized.

**Assay Procedures**
1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Leave 1 well for the blank, add 100 µl of Negative Control to the next 3 wells, then 100 µl of Positive Control to the following 2 wells. Add 100 µl of Sample Diluent into each of the rest of the wells, and then add 10 µl of specimen into each of the wells with added Sample Diluent.
3. Mix thoroughly by shaking on a vortex mixer for 10 seconds. Apply the plate seal, Incubate at 37°C for 45 minutes.
4. Wash 6 times (an automated microplate strip washer is recommended); strike the microtiter plate onto absorbent paper at the end of the last wash cycle.
5. Add 100 µl of Enzyme Conjugate Reagent into each well except for the blank well.
6. Repeat steps 3 and 4.
7. Add 50 µl of Substrate Solution, then 50 µl of Chromogen Solution into each well including the blank well. Gently mix and incubate at 37°C for 10 minutes without exposure to sunlight.
8. Add 50 µl of Stop Solution to each well. Mix thoroughly on a vortex mixer.
9. Immediately after mixing, read the absorbance of each well at 450 nm using 620 – 630 nm as the reference wavelength. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

**Important Note:**
1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

**Interpretation of results**
The specimen is positive when the absorbance ≥ the cut-off value, otherwise, the specimen is negative.

**Calculation of Results**
1. Test is valid only if absorbance of Positive Control ≥ 0.7, and absorbance of Negative Control ≤ 0.1.
2. Calculation of the cut-off value:
   - Cut-off value = 0.1 + mean absorbance of Negative Control replicates (in case the mean absorbance of Negative Control replicates < 0.05, use 0.05 instead of the actual mean)

**Performance Characteristics**
1. **Sensitivity**
   - The sensitivity reaches 100% (191/191)

2. **Specificity**
   - The specificity is 97.37% (556/571)

3. **Precision**
   - After 10 replicate tests, the precision was calculated to be ≤ 15%.

**Limitation**
1. This assay is only suited for aiding in the diagnosis of Toxo infected patients, not to be used to screen blood sources.
2. This assay is not suited for monitoring the therapeutic treatment for Tox infections.

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